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Identification of Serologic Markers for School-Aged Children With Congenital Rubella Syndrome

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Abstract

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Background—Congenital rubella syndrome (CRS) case identification is challenging in older children since laboratory markers of congenital rubella virus (RUBV) infection do not persist beyond age 12 months.

Methods—We enrolled children with CRS born between 1998 and 2003 and compared their immune responses to RUBV with those of their mothers and a group of similarly aged children without CRS. Demographic data and sera were collected. Sera were tested for anti–RUBV immunoglobulin G (IgG), IgG avidity, and IgG response to the 3 viral structural proteins (E1, E2, and C), reflected by immunoblot fluorescent signals.

Results—We enrolled 32 children with CRS, 31 mothers, and 62 children without CRS. The immunoblot signal strength to C and the ratio of the C signal to the RUBV-specific IgG concentration were higher ($P < .029$ for both) and the ratio of the E1 signal to the RUBV-specific IgG concentration lower ($P = .001$) in children with CRS, compared with their mothers. Compared with children without CRS, children with CRS had more RUBV-specific IgG ($P < .001$), a stronger C signal ($P < .001$), and a stronger E2 signal ($P \leq .001$). Two classification rules for children with versus children without CRS gave 100% specificity with >65% sensitivity.

Conclusions—This study was the first to establish classification rules for identifying CRS in school-aged children, using laboratory biomarkers. These biomarkers should allow improved burden of disease estimates and monitoring of CRS control programs.

Keywords

congenital rubella syndrome; CRS; biomarkers; rubella; serology; immune response

Rubella virus (RUBV) infection during pregnancy can lead to fetal infection and cause miscarriage, fetal death, or congenital rubella syndrome (CRS), which includes birth defects such as cataracts, sensorineural hearing loss, heart defects, and mental retardation [1]. Despite availability of safe, effective, and inexpensive vaccines, the World Health Organization (WHO) estimated that 103 000 infants with CRS were born in 2010, mainly in developing countries that have not introduced rubella vaccination [1]. In 1998, the Technical Advisory Group on Vaccine-Preventable Diseases of the Region of the Americas recommended that all countries in the region incorporate rubella virus (RUBV)–containing vaccine (RCV) into their childhood vaccination program [2, 3]. In 2011, the WHO recommended that all countries take the opportunity offered by accelerated measles control and elimination activities to introduce RCV [4]. By 2012, 132 (68%) of 194 WHO Member States included RCV in their routine immunization programs [5].

Determining the burden of CRS-related disabilities may inform decisions regarding vaccine introduction. The CRS incidence has been measured in follow-up studies of women infected with RUBV during pregnancy [6, 7], obtained through CRS surveillance targeting children <12 months of age [8–12]or estimated using mathematical models based on anti-RUBV immunoglobulin G (IgG) seroprevalence among child-bearing–aged women [13, 14]. Retrospective studies based on clinical diagnosis have provided evidence of the contribution of CRS to the burden of congenital deafness, visual impairment, and other birth defects [15–17].

Suspected CRS case identification can be challenging since clinical symptoms such as sensorineural hearing loss are often not clinically apparent in the first year of life. Since individual CRS-associated disabilities may be caused by other infectious agents or genetic factors, laboratory assays for confirming CRS diagnosis are required. Confirming acute RUBV infection includes direct virus detection and immunologic/serologic assays to detect the presence of anti-RUBV immunoglobulin M (IgM) [18]. These 2 methods of confirmation (biomarkers) are reliable until about 12 months of age in CRS cases. Identifying serologic markers of congenital RUBV infection that persist beyond 12 months of age would extend the period for laboratory confirmation of suspected CRS cases.

RUBV contains 2 envelope glycoproteins (E1 and E2) and 1 capsid protein (C), which have been studied as potential immunologic markers for CRS [1]. Immunologic studies have reported differences in the responses to these proteins between patients with CRS and individuals postnatally infected with RUBV. Among the latter group, mature immune responses have been characterized by development of high-avidity IgG antibodies to a neutralizing epitope on the E1 protein [19]. In contrast, immune responses of patients with CRS show persistent low-avidity IgG antibodies, with relatively low antibody signal to the E1 protein and higher signal to C protein in some studies [20]. Follow-up studies suggest that CRS-specific immune responses may persist into adulthood [21, 22].

São Paulo was the first Brazilian state to introduce universal childhood vaccination with measles-mumps-rubella vaccine, beginning in 1992 with mass vaccination of children aged 1–11 years. Rubella outbreaks with high incidence among young adults occurred in 1998–2000, and >133 confirmed rubella cases in pregnant women were reported in 1999 and 2000 [23]. Brazil developed a vaccination effort, using measles-rubella vaccine (containing Edmonston-Zagreb measles and RA 27/3 RUBV strains), to accelerate CRS prevention during 2000 and 2001. A second national rubella vaccination campaign, conducted in 2008, interrupted endemic rubella transmission; the last endemic CRS case occurred in 2009.

The goal of the current serologic study was to characterize the immune responses of school-aged children with CRS and compare these responses with those of their biological mothers and a group of similarly aged children without CRS. We hypothesized that specific immune responses, including the persistence of low-avidity IgG antibody, the level of the IgG anti-E1 signal, and the level of the IgG anti-C signal, would be associated with CRS.

METHODS

Study Period and Locations

Data collection took place from 2008 to 2011. The study was conducted in São Paulo State and included participation of tertiary care referral university hospitals and specialized centers providing services to deaf children in the cities of São Paulo, Bauru, Campinas, and Ribeirão Preto.

Study Population

Eligible children born between 1995 and 2003 with birth defects clinically compatible with CRS were identified through medical records of regional health departments in São Paulo

State, pediatric specialty services (cardiology, otolaryngology, ophthalmology, and fetal medicine) at 6 referral hospitals, and 2 centers providing services to deaf children in São Paulo state, described at the end of the text.

Case Definitions

CRS cases were classified as clinically compatible, probable, or laboratory confirmed. Clinically compatible CRS was defined as the documented presence at any time after birth of either 2 major signs or symptoms (ie, cataracts/congenital glaucoma, pigmentary retinopathy, hearing impairment, or congenital heart disease [eg, patent ductus arteriosus or peripheral pulmonic stenosis]) or 1 major and 2 minor signs or symptoms (ie, purpura, hepatosplenomegaly, jaundice, microcephaly, developmental delay, meningoencephalitis, or radiolucent bone disease), without documented evidence of alternative diagnosis.

Probable CRS cases were children with clinically compatible but not laboratory confirmed CRS.

Laboratory-confirmed CRS cases included (1) individuals with clinically compatible CRS with documented positive results of RUBV-specific IgM serologic testing or detection of RUBV by polymerase chain reaction or virus isolation within the first year of life and (2) children with signs or symptoms associated with CRS, whether or not they met the clinically compatible CRS case definition, whose mothers had laboratory-confirmed RUBV infection (defined as positive results of RUBV-specific IgM serologic testing) during pregnancy [24].

Comparison Groups

Mothers of Children With CRS—Mothers of enrolled children with CRS were enrolled as one comparison group. The mothers' immune responses were due to postnatal RUBV infection during pregnancy. It was assumed that children with CRS were infected with the same RUBV strain as their mothers. Thus, testing and analysis of results from pairs of mothers and children with CRS controlled exactly for the infecting virus.

Children Without CRS—We enrolled a second comparison group of children born between 1995 and 2003 with no history of signs and symptoms compatible with CRS and who were vaccinated against rubella (some may have also had undiagnosed postnatal RUBV infection). These children were recruited from outpatient clinics where children with CRS were identified, were matched by age to children with CRS, and were unrelated to children with CRS.

Inclusion and Exclusion Criteria

Children who met the clinically compatible, probable, or laboratory-confirmed CRS case definitions were considered for enrollment in the study. Children who were identified as having had congenital RUBV infection without defects associated with CRS were excluded.

Data Collection

We used standardized questionnaires to collect demographic data (ie, age, sex, and race/ethnicity), history of RUBV infection and related clinical information, and rubella vaccination status. We reviewed medical charts for laboratory test results, if available.

Laboratory Testing

Sample Collection and Transport—At the time of demographic data collection, approximately 3 mL of venous blood was collected from each enrollee. Two aliquots of sera were prepared at study sites and maintained at −20°C or 4°C–8°C until shipment to Adolfo Lutz Institute in São Paulo, where they were stored at −20°C. One aliquot was shipped to the Centers for Diseases Control and Prevention (CDC; Atlanta, Georgia) and stored at −70°C. We conducted serologic testing on samples from all study subjects.

IgG Enzyme-Linked Immunosorbent Assay (ELISA) for RUBV-Specific Antibody—RUBV-specific IgG antibody concentrations (expressed as international units [IU] per milliliter) were determined using the Rubella IgG ELISA II system according to manufacturer's instructions (Wampole Laboratories, Princeton, New Jersey). The OD ratio was calculated by dividing the specimen OD by the cutoff value supplied by manufacturer. Specimens with OD ratios of >2.2 were diluted with kit dilution buffer and RUBV-specific IgG antibody concentrations were determined from the diluted serum.

Measurement of RUBV-Specific IgG Antibody Avidity—Absorbance of each serum specimen was tested using the RUBV IgG ELISA II system with 35 mM diethylamine (DEA) added to washing buffer to elute antibodies with low avidity. Avidity was calculated as the ratio of the absorbance with and the absorbance without DEA, and the ratio was converted to a percentage. The acceptable range of the total IgG antibody concentration for determination of avidity was 10–70 IU/mL; sera with total IgG concentrations of >70 IU/mL were diluted to <70 IU/mL, using an ELISA kit dilution buffer before testing [25]. Serum samples were tested in duplicate for avidity, and each run included both a high-avidity and a low-avidity control serum.

Quantifying Immune Response to RUBV Antigens by Immunoblot—The amount of IgG antibody in each serum specimen binding to 3 individual RUBV antigens (E1, E2, and C) was quantified using the Nupage system (Invitrogen, Carlsbad, California). Briefly, 25 μg of RUBV antigen, highly purified RUBV strain HPV77 (Meridian, Memphis, Tennessee; catalog no. 6123), was denatured in loading buffer containing DTT (Invitrogen), heated for 5 minutes at 95°C, and electrophoresed through 10% Bis-Tris gel (Invitrogen) in the presence of antioxidant. Proteins were transferred onto polyvinylidene fluoride membranes according to the manufacturer's instructions (iBlot Western Blotting System, Invitrogen). Membranes were placed in a Mini-Protean II Multi Screen system (BioRad, Hercules, California) and blocked with phosphate-buffered saline (PBS) containing 5% skim milk, 0.1% Tween 20, and 0.1% fetal bovine serum. Separate areas of the membrane were incubated with 4 μL, 2 μL, 1 μL, and 0.5 μL of serum in 200 μL of blocking buffer for 90 minutes. Membranes were washed 4 times with PBS containing 0.1% Tween 20, incubated with 0.67 μg/mL fluorescent goat anti-human IgG-633 (Invitrogen) in block buffer, and

washed 4 times. Fluorescent signal was measured with a Typhoon 9410 variable mode imager (GE Healthcare, Piscataway, New Jersey). Signal intensity in each band (E1, E2, and C) for each amount of serum was quantified using ImageQuant software (GE Healthcare, Piscataway, New Jersey).

We evaluated reproducibility of the quantitative immunoblot method in 180 repetitions using the standard control serum. The percentage and standard deviation of fluorescence signal measured for each RUBV antigen, as a percentage of total measured signal for all 3 antigens, was 27.2% ± 4.9% for E1, 51.3% ± 4.6% for E2, and 21.4% ± 4.8% for C. For sera from study participants, results of 3 separate immunoblots were averaged for each serum to reduce error, compared with single immunoblots. A linear fit of signal strength at the 4 serum concentrations was then determined by least squares. The final signal strength directed toward each RUBV antigen was then calculated as the signal strength for 2 μL of serum from the fitted line. To simplify analysis, signal strength was expressed as the ratio of the corrected signal strength to the corrected signal strength to E1 in 2 μL of the standard control serum (eg, ratios of >1.0 indicate stronger signal than that of the standard control serum to the E1 protein, while ratios <1.0 indicate weaker signal; Figure 1).

Statistical Analysis

Data were entered using SPSS for Windows (release 11, SPSS) and analyzed in SAS 9.3 (SAS Institute, Cary, North Carolina) and R 3.01 [26]. Potential biomarkers of CRS that were evaluated included total RUBV-specific IgG; IgG avidity; immunoblot signal strength to E1, E2, and C; and the ratio of E1, E2, or C signal strength to RUBV-specific IgG concentration. For pairs of children with CRS and their mothers, we conducted paired analyses for differences in biomarker distribution, using the nonparametric sign test. We tested for differences in the distribution of biomarkers between children with and children without CRS, using the Wilcoxon rank sum test. *P* values of < .05 were considered statistically significant.

To evaluate the ability of different biomarkers to differentiate children with from children without CRS, we calculated receiver operating characteristic (ROC) curves [26], which plot sensitivity against [1 – specificity] for every cutoff in the range of the observed biomarker values. We calculated the area under the ROC curve (AUC), which is considered a measure of diagnostic accuracy. For biomarkers with high AUCs, we defined a case-classification rule based on a marker-specific cutoff. We then estimated sensitivity and specificity and the corresponding 95% Wilson confidence interval for each rule.

Ethical Issues

The study protocol was approved by ethical review boards at participating institutions in São Paulo state, the São Paulo State Health Department, the Brazilian National Committee for Ethics in Research, the Pan American Health Organization, and the CDC. Informed consent was obtained from all study participants.

RESULTS

After reviewing medical records for 317 children aged 6–14 years detected through participating institutions, we identified 30 clinically compatible CRS cases, of which 13 were laboratory confirmed. Follow-up investigation of 225 suspected CRS cases reported to the São Paulo State Health Department and 88 suspected cases identified from laboratory records of the state measles/rubella reference laboratory at the Adolfo Lutz Institute identified 2 clinically compatible, laboratory-confirmed CRS cases. Thirty-one mothers of children with CRS and 62 age-matched children without CRS were enrolled in the comparison groups.

Among the 32 children with CRS, 11 (34.4%) were female, the median age was 10.5 years (range, 6–14 years), 28 (87.5%) had a history of at least 1 postnatal dose of rubella vaccine, and 15 (46.9%) had laboratory-confirmed CRS. Deafness was the most common disability (87.5%), followed by cardiac defects (71.9%) and cataracts (43.8%; Table 1). We compared bio-marker distributions of laboratory-confirmed and clinically confirmed cases and found no differences (Table 2). Twenty-one enrolled mothers (67.0%) reported clinical symptoms of rubella or a diagnosis of rubella during pregnancy; 16 (51.6%) reported a history of ≥1 rubella vaccine dose after the CRS-affected pregnancy. Among 62 children in the comparison group, 33 (53.2%) were female, the median age was 10 years (range, 5–13 years), and 58 (93.6%) had history of receiving ≥1 rubella vaccine dose. Children with and children without CRS were not significantly different when comparing sex ($P = .08$, by the χ^2 test), age ($P = .52$, by the Wilcoxon rank sum test), and history of reported rubella vaccination ($P = .44$, by the Fisher exact test).

In matched analysis of case-mother pairs, children with CRS had significantly lower IgG antibody avidity than their mothers, despite similar levels of total RUBV-specific IgG antibody (Table 3). Immunoblot reactivity (signal strength) with C protein among children with CRS was higher than that among their mothers, as was the ratio of the C protein signal to the RUBV-specific IgG antibody concentration ($P \leq .029$). The E1 signal strength among children with CRS may have been lower than that among their mothers (the difference was not statistically significant; $P = .071$), whereas the ratio of the E1 signal strength to the total IgG antibody concentration was significantly lower in children with CRS ($P = .001$; Table 3).

Children with CRS had significantly higher concentrations of RUBV-specific IgG antibody than children without CRS ($P > .001$); however, antibody avidity was similar in both groups ($P = .07$; Table 3 and Figure 2). Immune responses among children with CRS exhibited higher signals to C protein ($P \leq .001$) and to E2 protein ($P \leq .001$) than did those among children in the comparison group. Ratios of the antigen-specific signal strength to the RUBV-specific IgG antibody concentration among case patients were significantly higher than those among children without CRS for E1/IgG ($P \leq .001$), E2/IgG ($P \leq .001$), and C/IgG ($P \leq .001$; Table 3). The E1 signal strength and avidity were similar for children with and children without CRS (Figure 2).

The ROC curves for biomarkers indicated that C signal strength was the serologic biomarker that best differentiated children with from children without CRS (AUC, 0.91; Figure 3). A rule classifying children with a C signal strength of >0.05 as CRS cases resulted in a sensitivity of 84.4% (95% confidence interval [CI], 68.3%–93.1%) and specificity of 79% (95% CI, 67.4%–87.3%; Table 4).

When standardized for total IgG, the ROC for E1 signal strength resulted in an AUC of 0.92, and the ROC for C signal strength yielded an AUC of 0.90. A rule classifying children with an E1/IgG ratio of <0.01 as CRS cases resulted in a sensitivity of 75.0% (95% CI, 57.9%–86.8%) and a specificity of 100% (95% CI, 94.2%–100%). A rule classifying children with a C/IgG ratio of > 0.003 as CRS cases resulted in a sensitivity of 68.8% (95% CI, 51.4%–82.1%) and a specificity of 100% (95% CI, 94.2%–100%).

DISCUSSION

This is the largest study to date examining RUBV-specific immune responses of school-aged children with CRS and the first to document useful classification rules for CRS among children in this age range, using laboratory biomarkers. The quantitative immunoblot method, which measured the IgG response to 3 viral structural proteins (E1, E2, and C) as reflected by fluores-cent signals on immunoblots, allowed identification of markers of CRS in children aged >12 months, when the sensitivity of current laboratory confirmation methods declines. These bio-markers can improve disease burden estimates and monitoring of CRS control programs, especially in settings where CRS surveillance is challenging.

The use of 2 comparison groups in this study was valuable. Most of the children with and without CRS were vaccinated; however, despite additional exposure(s) through vaccination, the CRS-specific immune response persisted. Furthermore, comparisons of immune responses of children with CRS to those of their mothers showed that some biomarkers that differentiate between children with and children without CRS also significantly differentiated children with CRS from their mothers. Ratios of antigen-specific signal to total RUBV-specific IgG antibody concentrations (eg, E1/IgG and C/IgG), which were significant using both comparison groups in this study, warrant further investigation. The usefulness of these 2 biomarkers should be tested in further studies to develop methods for estimating the CRS burden in populations with specific disabilities, such as hearing impairment.

Comparison of results from our study and those from other studies was useful. Immune responses among mothers were consistent with those of individuals postnatally infected with RUBV [20]. Total IgG titers among mothers of children with CRS in our study (mean, 211 IU/mL) were higher than those reported among naturally infected 50–59-year-old individuals (mean, 71 IU/mL) [26] and were consistent with more recent infection (6–14 years ago). In this study, IgG antibodies with relatively low avidity and the reactivity to RUBV E1 protein among individuals with CRS, compared with those of non-CRS groups, were consistent with previous results, which have also suggested that differences in the immune response in CRS persist into adulthood [27]. A study of 24 children with CRS (age, 1–34 years) and 15 individuals without CRS (mothers or children with postnatal rubella)

found that patients with CRS had lower-avidity IgG to RUBV and lower avidity to renatured E1 protein than the non-CRS comparison group [20]. Low-avidity antibodies among children with CRS, compared with cases of postnatal infection, were also reported in another study [28]. Our finding of low avidity in children with CRS, compared with their mothers, is consistent. Lower avidity in persons with vaccine-derived immunity, compared with those who had post-natal wild-type infection, is expected [25] and consistent with our finding of a lower strength of association of avidity between children with and without CRS. Further studies of the immune response of CRS cases versus those of vaccinated persons (eg, cell-mediated immune response to RUBV infection) would be interesting. In another study, involving 7 children with CRS (age range, 1 month to 9 years) and 11 children without CRS (age range, 7–13 years), an association between CRS and stronger reactivity to E2 was found [29].In our study, we found no difference between E2 reactivity when comparing children with CRS and their mothers, while E2 reactivity among children with CRS versus children without CRS was significant, even after control for total IgG. The strong association between CRS cases and C signal strength found here has not been consistently reported [29].

The reagents and methods used for immunoblots are commercially available, and the commercial immunoassay kits and methods we used to quantify RUBV-specific IgG concentration and avidity are similar to those used in other studies [20, 30]. This should allow our results to be reliably reproduced elsewhere. The reports of detectable E1, E2, and C signal being characteristic of postnatal rubella cases are not consistent with our data, especially with respect to C signal [27]. This could be because our results are quantitative rather than qualitative or because enrollees in our study were evaluated 6–14 years after infection. Our preliminary results with nonreducing blots indicate that differences in antigens on nonreducing immunoblots versus reducing immunoblots is not the explanation for lack of consistency with other reports (data not shown).

This study has some limitations. The prevalence of cardiac defects among enrolled CRS cases was more prevalent than commonly seen among CRS survivors, likely because of enrollment bias. An association between cardiac defects and immune response would restrict our results. Enrollment of CRS cases was mostly from referral centers where affected individuals received care for defects due to CRS. While we did not include a comparison group of children with disabilities unrelated to CRS, a reasonable biologic assumption is that such persons will respond to postnatal RUBV infection in the same way as individuals without disability. Since we enrolled children without CRS on the basis of lack of disability, it is possible that some children without CRS were infected with RUBV in utero without having defects. The unrecognized prevalence of such congenital RUBV infection among children without CRS is estimated to be low and should not influence our results.

Documenting the CRS burden is important for evaluating immunization strategies to eliminate rubella and CRS [31, 32] and yields data that can help support verification of rubella and CRS elimination [9], such as activities currently underway in the WHO Region of the Americas [31, 33]. Estimating the CRS burden and the costs of long-term care for children born with disabilities due to CRS should be considered in countries planning to introduce RCV [34]. The immunoblot method described could be used to estimate the

proportion of disabilities due to CRS among a group (or groups) of children with a specific disability or disabilities, such as hearing impairment. The availability of support for introduction of RUBV-containing vaccines should provide additional incentive for countries to evaluate their CRS burden.

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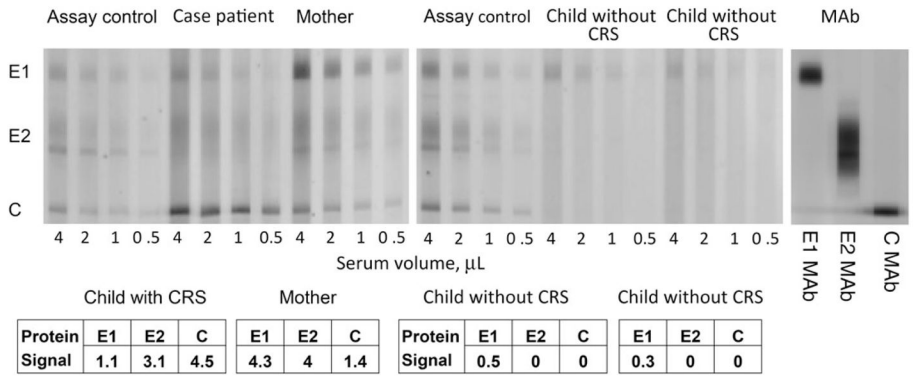
STUDY SITES AND STUDY GROUP MEMBERS

The study included the participation of tertiary care referral university hospitals and specialized centers providing services to deaf children in the following cities of São Paulo State: São Paulo, Bauru, Campinas, and Ribeirão Preto.

The settings from which children with CRS, their mothers, and unrelated children were selected were as follows: Instituto da Criança, Hospital das Clínicas, Universidade de São

Paulo (USP); Hospital São Paulo/Universidade Federal de São Paulo; Faculdade de Ciências Médicas da Santa Casa; Faculdade de Ciências Médicas da Universidade Estadual de Campinas; Hospital da Clínicas da USP, Ribeirão Preto; University Hospital of Botucatu; Audiology Research Center, Hospital for Rehabilitation of Cranofacial Abnormalities, USP, Bauru; Divisão de Educação e Reabilitação dos Distúrbios da Comunicação, Pontificia Universidade Católica de São Paulo (PUCSP); and Division of Otorhinolaryngology, USP Medical School.

Members of the CRS Biomarker Study Group are as follows: Terri B. Hyde, Susan E. Reef, Joseph P. Icenogle, LiJuan Hao, Brendan Flannery, Qi Zheng, and Kathleen Wannemuehler (CDC); Telma Regina Marques Pinto Carvalhanas, Flavia Ciccone, and Helena Keico Sato (São Paulo State Health Department); Cristiana M. Toscano, Jon K. Andrus, and Carlos Castillo-Solorzano (Pan American Health Organization); Heloisa de Sousa Marques (Children's Institute, University Hospital, USP); Lily Yin Weckx and Alessandra Ramos Souza Hospital São Paulo, Federal University of São Paulo); Marco Aurélio Sáfadi (School of Medical Sciences of Santa Casa); Eliane Moraes (School of Medical Sciences, University of São Paulo, Campinas); Marisa Mussi-Pinhata and Fabiana Rezende Amaral (Unviersity Hospital, USP, Ribeirão Preto); Jaime Olbrich Neto (University Hospital of Botucatu); Maria Cecilia Bevilacqua and Regina B. Amantini (Audiology Research Center, Hospital for Rehabilitation of Cranofacial Abnormalities, USP, Bauru); Alfredo Tabith Junior (Division of Education and Rehabilitation for Communication Disturbances, Catholic University of São Paulo); Tatiana Alves Monteiro and Ricardo Ferreira Bento (Division of Otorhinolaryngology, USP Medical School); and Ana Maria Sardinha Afonso, Cristina Adelaide Figueiredo, and Sueli Pires Curti (Adolfo Lutz Institute).



	Child with CRS			Mother			Child without CRS				Child without CRS		
Protein	E1	E2	C	E1	E2	C	Protein	E1	E2	C	E1	E2	C
Signal	1.1	3.1	4.5	4.3	4	1.4	Signal	0.5	0	0	0.3	0	0

Figure 1.

Representative immunoblots made by the Typhoon 9410 imager for serum samples from a child with congenital rubella syndrome (CRS), the mother of the child, and 2 children without CRS, as well as assay controls. The amount of serum used in each portion of the blot is indicated. A scan of an immunoblot using monoclonal antibodies (MAb) to the individual proteins is included at right for comparison. The amount of the protein signal is given in E1 units, which is defined as the amount of E1 signal obtained with 2 µL of assay control serum run in parallel with study sera. Note that signal for each serum was normalized to the control serum run on the same blot. For example, the signal strength of the E1 bands for the children without CRS are seen by inspection to both be less than that of the E1 band in the control for that run. Data in the table are from the immunoblots shown, and these immunoblots are one of 3 replicates which were averaged for these sera to provide the results used for comparisons made in this study.

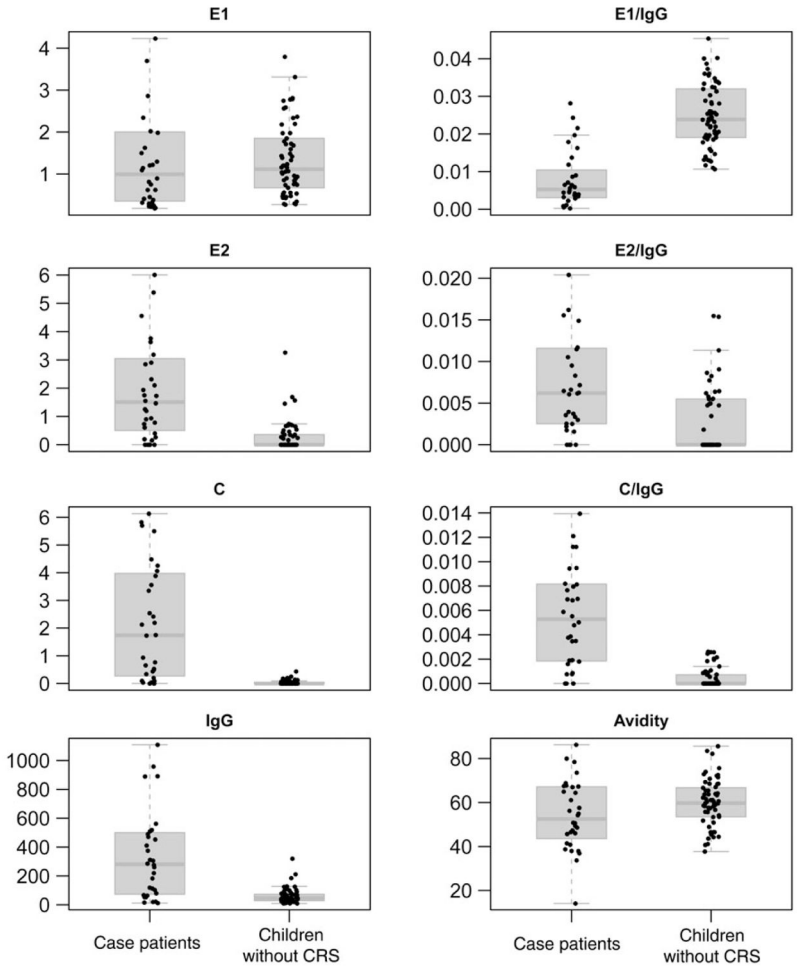


Figure 2.

Box plots showing the distribution of each biomarker for children with and without congenital rubella syndrome (CRS), São Paulo, Brazil, 2008–2011. Abbreviation: IgG, immunoglobulin G.

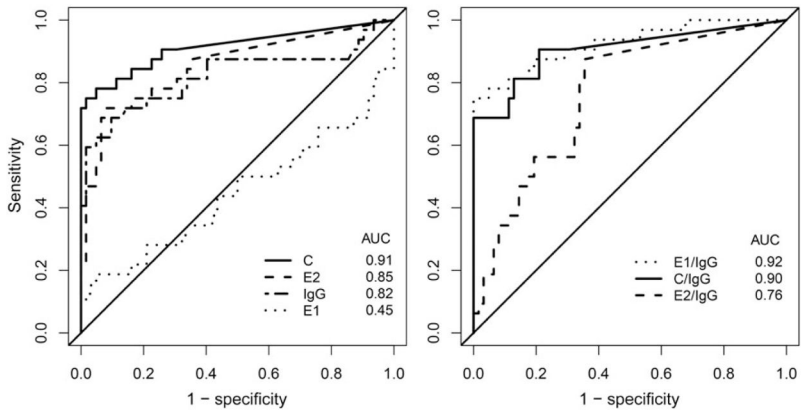


Figure 3.

Receiver operating characteristic curves and estimates of areas under the receiver operating characteristic curve (AUCs) for each biomarker among children with and without congenital rubella syndrome (CRS), São Paulo, Brazil, 2008–2011. Abbreviation: IgG, immunoglobulin G.

Table 1

Characteristics of 32 Children With Congenital Rubella Syndrome (CRS), São Paulo, Brazil, 2008–2011

Characteristic	Children
Age, y	10.5 (6–14)
Female sex	11 (34.4)
Previously vaccinated against rubella	
Overall	28 (87.5)
Received 1 dose	9 (28.1)
Received ≥2 doses	19 (59.4)
Reported history of postnatal rubella illness	0 (0)
CRS case classification	
Laboratory confirmed	15 (46.9)
Not laboratory confirmed	17 (53.1)
Major sign	
Deafness	28 (87.5)
Cataracts	14 (43.8)
Glaucoma	6 (18.8)
Retinopathy	7 (21.9)
Cardiac defect	23 (71.9)
Minor sign	
Mental retardation	12 (37.5)
Microcephaly	6 (18.8)
Hepatosplenomegaly	4 (12.5)
Icterus	4 (12.5)
Purpura	3 (9.4)

Data are no. (%) of case patients or median value (range).

Table 2

Comparison of Total Rubella Virus (RUBV)–Specific Immunoglobulin G (IgG) Concentration, RUBV-Specific IgG Avidity, and IgG Signal Strength to Individual RUBV Antigens Among 15 Children With Laboratory-Confirmed Congenital Rubella Syndrome (CRS) and 17 With Clinically Confirmed CRS, São Paulo, Brazil, 2008–2011

Serologic Marker	Laboratory-Confirmed CRS, Median (IQR)	Clinically Confirmed CRS, Median (IQR)	*P* Value[a]
RUBV-specific IgG, IU/mL	286.0 (64.0–490.0)	276.0 (103.0–510.0)	.78
IgG avidity, %	48.6 (41.5–67.3)	55.0 (46.0–67.0)	.47
E1 signal[b]	0.620 (0.248–2.020)	1.143 (0.620–1.983)	.36
E2 signal[b]	1.255 (0.194–2.846)	1.727 (0.782–3.186)	.30
C signal[b]	1.751 (0.338–3.558)	0.763 (0.091–4.255)	.91
E1 signal/IgG concentration	0.005 (0.003–0.009)	0.006 (0.003–0.012)	.79
E2 signal/IgG concentration	0.004 (0.002–0.100)	0.007 (0.004–0.012)	.20
C signal/IgG concentration	0.007 (0.004–0.009)	0.003 (0.002–0.008)	.13

Abbreviation: IQR, interquartile range.

[a] By the nonparametric sign test.

[b] The amount of signal in 2 μL of test serum is given in E1 units, the amount of E1 signal obtained with 2 μL of assay control serum.

Table 3

Comparison of Total Rubella Virus (RUBV)-Specific Immunoglobulin G (IgG) Concentration, RUBV-Specific IgG Avidity, and IgG Signal Strength to Individual RUBV Antigens for Sera From Children With Congenital Rubella Syndrome (CRS), Mothers of Children, and Age-Matched Children Without CRS, São Paulo, Brazil, 2008–2011

Serologic Marker	Children With CRS, Median (IQR)	Mothers of Children With CRS, Median (IQR)	*P* Value[a]	Children Without CRS, Median (IQR)	*P* Value[b]
RUBV-specific IgG, IU/mL	281.0 (73.5–500.0)	221.0 (75.0–311.0)	.281	47.5 (29.0–73.0)	<.001
IgG avidity, %	52.5 (43.6–67.2)	75.7 (65.2–79.4)	<.001	59.8 (53.5–66.7)	.07
E1 signal[c]	0.994 (0.354–2.002)	1.829 (1.240–2.856)	.071	1.1 (0.7–1.8)	.40
E2 signal[c]	1.508 (0.505–3.045)	2.273 (0.771–5.308)	.473	0.0 (0.0–0.36)	<.001
C signal[c]	1.739 (0.269–3.973)	0.314 (0.177–0.639)	.029	0.0 (0.0–0.04)	<.001
E1 signal/IgG concentration	0.005 (0.003–0.010)	0.012 (0.006–0.018)	.001	0.024 (0.019–0.032)	<.001
E2 signal/IgG concentration	0.006 (0.003–0.012)	0.011 (0.007–0.0021)	.029	0.000 (0.000–0.005)	<.001
C signal/IgG concentration	0.005 (0.002–0.008)	0.002 (0.001–0.003)	.029	0.000 (0.000–0.001)	<.001

Abbreviation: IQR, interquartile range.

[a] By the nonparametric sign test, for comparison of children with CRS and their mothers. One pair was omitted because the mother was not enrolled.

[b] By the Wilcoxon rank sum test, for comparison of children with and without CRS.

[c] The amount of signal in 2 μL of test serum is given in E1 units, the amount of E1 signal obtained with 2 μL of assay control serum.

Table 4

Sensitivity and Specificity of Biomarkers for Congenital Rubella Syndrome (CRS) Among 32 Children With CRS and 62 Unrelated Age-Matched Children Without CRS, São Paulo, Brazil, 2008–2011

Biomarker	Case Classification Rule	Sensitivity (95% CI)	Specificity (95% CI)
IgG	>100	71.9 (54.6–84.4)	85.5 (74.7–92.2)
E2	>1	59.4 (42.3–74.5)	93.6 (84.6–97.5)
C	>0.05	84.4 (68.3–93.1)	79.0 (67.4–87.3)
E1/IgG	<0.01	75.0 (57.9–86.8)	100 (94.2–100)
E2/IgG	>0.005	56.3 (39.3–71.8)	74.2 (62.1–83.5)
C/IgG	>0.003	68.8 (51.4–82.1)	100 (94.2–100)

E1 and avidity are not included because these biomarkers are poor at differentiating between children with and children without CRS.

Abbreviations: CI, confidence interval; IgG, immunoglobulin G.